CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA

MILBEMECTIN

Chemical Code # 5763, Tolerance # 52855

4/27/01

I. DATA GAP STATUS

Combined, rat:	No data gap; no adverse effect
Chronic toxicity, dog:	No data gap; no adverse effect
Oncogenicity, mouse:	No data gap; no adverse effect
Reproduction, rat:	No data gap; **possible adverse effect**
Teratology, rat:	No data gap; no adverse effect
Teratology, rabbit:	No data gap; no adverse effect
Gene mutation:	No data gap; no adverse effect
Chromosome effects:	No data gap; no adverse effect
DNA Damage:	No data gap; no adverse effect
Neurotoxicity:	Not required at this time

Toxicology one-liners are attached.

All record numbers through 176395 were examined.
** indicates an acceptable study.
Bold face indicates a possible adverse effect.
indicates a study on file but not yet reviewed.
File name: T184442.doc
Original 4/27/01, P. Leung

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. Individual worksheets may contain additional effects.

COMBINED, RAT

018; 176389; "Concurrent Study of Chronic Toxicity and Carcinogenicity of E-187 in Rats"; (Matsunuma, N.; Sankyo Co., Ltd. Laboratory Animal Science and Toxicology Laboratories, Fukuroi, Shizuoka, Japan.; Report No. TR136-028; 06/16/89). F344 rats (80 rats/sex/group) were fed E-187 technical (Lot #s MA-1220, MA-1220-2, MA-1220-3, MA-1220-4, MA-1220-6, and MA-1220-7; purity ca. 94.5 % a.i.); in the diet at concentrations of 0, 15, 150, 750 ppm (1500 ppm up to week 7)((M): 0, 0.71, 6.81, 32.64 (120.91 at 1500 ppm) mg/kg/day, (F): 0, 0.92, 8.77, 44.41 (132.39 at 1500 ppm) mg/kg/day) for 104 weeks. Symptoms such as stained eyelids, and marked overgrowth of incisors were observed at the beginning of administration at 1500 ppm. Animals in both sexes at 750 ppm showed a high incidence of thin hair and enlargement of hair follicles. The body weights of females were slightly suppressed from week 78 till the end of the administration at HDT. Increases in the relative weights of liver and kidneys were consistently observed in both sexes at HDT. Incidence of chronic nephrosis (moderate) and reduction in mineral deposition in the cortico-medullar junction were observed in the males and females, respectively at HDT. There were no increases in non-neoplastic and neoplastic lesions, abnormalities in food efficiency, urinary changes, or ophthalmological changes observed during the study that could be attributed to administration of the test material. Survival was unaffected by the administration. **No adverse effect indicated. Chronic NOEL (M/F): 150 ppm (M: 6.81 mg/kg/day, F: 8.77 mg/kg/day) (based on reduced body weight and thinning hair at 750 ppm); **Study acceptable.** (Eya, 12/26/00).

CHRONIC TOXICITY, DOG

014; 176383; "E-187: 12-Month Oral Chronic Toxicity Study in Dogs"; (Ebino, K.; The Institute of Environmental Toxicology, Kodaira City, Tokyo 187, Japan.; Laboratory Project ID No. IET 85-0147; 03/27/89). Forty-eight Beagle dogs (6/sex/group) were administered orally E-187 Technical (Lot # MA-22-2; 95.6 % a.i. [A3, 21.8 %; A4, 73.8 %]) by gelatin capsule at doses of 0, 3, 10, 30 mg/kg/day for 1 year. No mortality was observed during the study. No treatment-related abnormalities were found in any of the parameters in either sex at 3 mg/kg/day. The body weights of female were depressed in 10 mg/kg/day group from week 13, and at 30 mg/kg/day from week 1. The mean weights of female were 0.9 and 1.6 kg lower than the control value in the 10 and 30 mg/kg/day treatment groups, respectively, at week 52. The absolute and relative liver weights were significantly increased in males at 10 and 30 mg/kg/day. Other observations reported at 30 mg/kg/day dose level were lower food consumption, incidences of vomiting, sedation, staggering gait, tremors, and salivation in the females. Significant increases in cholesterol and calcium values were observed in the males at weeks 26 and 52. Also, sedation and staggering gait were observed in one male. **No adverse effect. No abnormal histopathological changes were detected in the treatment groups. Chronic NOEL (M/F): 10 mg/kg/day (based on clinical signs at 30 mg/kg/day); Study **acceptable**. (Eya, 11/08/00).

ONCOGENICITY, MOUSE

**015; 176384; "E-187: Oncogenicity Study in Mice"; (Maita, K.; The Institute of Environmental Toxicology, Kodaira City, Tokyo 187, Japan.; Laboratory Project ID No. IET 85-0148; 06/12/89). Four hundred-eighty SPF ICR (Crj:CD-1) mice (60/sex/group) were fed E-187 Technical (Lot # MA-1220; purity 94.0 % a.i.) in the diet at concentrations of 0, 20, 200, or 2000 ppm ((M): 0, 1.95, 18.9, 193 mg/kg/day, (F): 0, 1.97, 19.6, 231 mg/kg/day, respectively) for 96 weeks. After 52 weeks of

treatment, 10 mice/sex/group were subjected to interim sacrifice. There were no significant increases in the incidence of neoplastic lesions in the treated groups. No toxic effects related to the test substance administration were observed at 20 and 200 ppm concentrations. At 2000 ppm, both sexes showed significantly increased incidences of elongated incisor(s), and decrease or a tendency of body weight reduction. Females exhibited significant reduction of food consumption and body size, and emaciation at 2000 ppm. **No adverse effect indicated. Chronic NOEL (M/F):** 200 ppm (M: 18.9 mg/kg/day, F: 19.6 mg/kg/day) (based on body weight reduction at 2000 ppm); **Study acceptable.** (Eya, 11/15/00).

REPRODUCTION, RAT

**** 017; 176388**; "Two-Generation Reproduction Study in Rats with E-187"; (Kaneda, M.; The Institute of Environmental Toxicology, Kodaira City, Tokyo 187, Japan; Laboratory Project ID No. IET 85-0152; 05/30/88); Twenty-four rats/sex/group (F0) and (F1) were dosed orally in the diet with 0, 50, 200 and 800 ppm of E-187 Technical (Lot # MA-7, and 8; purity 93.2 %) for two generations. The treatment periods included 10 weeks prior to mating for F0 and 11 weeks prior to mating for F1 generation, and treatment continued during mating period and post-mating interval until sacrifice. Mated females were continued to be treated during the gestation, lactation and post-weaning periods until sacrifice. Significant depression of body weight in F0 and F1 females especially in weeks 1-2, and reduction in food consumption during lactation of F1 were observed at 800 ppm. Blotted fur on the dorsal lumbar region in F0 and F1 females, and reduction in food consumption in F1 males were also observed at this highest dose tested. Examination of the offsprings at 800 ppm revealed lower body weights in F1 and F2 pups, reduction in number and lower viability index of F2 pups on lactation day 0. **Possible adverse effect indicated. Parental Systemic NOEL: 200 ppm** (10 wk. mean, p. 45; (M) F0: 13.4, F1: 17.4 mg/kg/day;(F) F0: 14.8, F1: 18.8 mg/kg/day, due to reduction of food intake and body weight of F0 and F1 at HDT; **Reproductive NOEL: 800 ppm** (10 wk mean for (F) F0+F1; ca. 68.1 mg/kg/day, due to lack of adverse effects at HDT on reproductive parameters, i.e., mating, fertility, gestation indices, and duration of gestation). **Developmental NOEL: 200 ppm** (10 wk. mean for (F) F0+F1 ca.16.8 mg/kg/day, due to lower body weights of F1 and F2 pups, and reduction in viability of F2 at HDT). **Study acceptable.** (Eya, 12/11/00).

TERATOLOGY, RAT

** 016; 176385; "Teratogenicity Study in Rats with E-187" (M. Kaneda; The Institute of Environmental Toxicology, Tokyo 187, Japan; Laboratory Project ID No.: 85-0134). Twenty-four mated females/group were treated by stomach tube with 0, 6, 20, or 60 mg/kg/day of E-187 Technical (Lot # MA-7, 8, 93.2 % a.i.) from day 6-15 of gestation. No adverse effects were observed in the maternal rats in the parameters examined at 6 and 20 mg/kg/day. At 60 mg/kg/day dose level, the body weight gain during the gestation days 8-12, and food consumption during the gestation days 6-12 were significantly depressed compared to the controls. There were no other clinical signs or deaths during the treatment. Also, no adverse effects were observed from the fetuses in the parameters examined such as mortality, body weights, and malformations in any of the treatment groups. **No adverse effects indicated. Maternal NOEL:** 20 mg/kg/day (based on the reduced body weight gain and food consumption at 60 mg/kg/day); **Developmental NOEL:** 60 mg/kg/day (based on lack of treatment-related effects at HDT). **Study acceptable.** (Eya, 11/21/00)

TERATOLOGY, RABBIT

016; 176386; "Administration Study of E-187 in Rabbits During the Period of Organogenesis of Fetuses" (Tanase, H.; Sankyo Company, Ltd., Laboratory Animal Science and Toxicology Laboratories, Fukuroi, Shizuoka, Japan; Report No.: TR134-170, 1/26/88). Fourteen to 19 mated females/group were treated

orally via rubber catheter with 0, 160, 400, or 1000 mg/kg/day of E-187 (Lot # MA-22-2, 95.6 % % a.i.) from gestation days 6-18. Administration by gavage resulted in reductions in food intake and body weight in 2/14, 3/14, 5/17 dams at 160, 400, and 1000 mg/kg/day dose levels, respectively. From the dams which were affected, 1/2, 1/3, 4/5 dams in 160, 400, and 1000 mg/kg/day groups had abortions, and the development of fetuses in the remaining dams affected were markedly suppressed. Mean body weights or food intake in the treatment groups, which did not abort fetuses (13/14, 13/14, 13/17 dams at 160, 400, and 1000 mg/kg/day dose levels), were comparable to the control group. There were no treatment-related mortalities and teratogenicity observed in embryos or fetuses in the treated groups. The reduction in body weight of the fetuses from the affected dams in all 3 treatment groups were secondary effects due to the marked reduction of maternal food uptake. **No adverse effects. Maternal NOEL:** < 160 mg/kg/day (based on reduction of food intake and body weight of 2/14 dams at 160 mg/kg/day dose level); **Developmental NOEL:** 1000 mg/kg/day (based on lack of treatment-related effects observed at 1000 mg/kg/day); **Study unacceptable** due to the lowest dose used exceeding the maternal NOEL (Eya, 11/29/00).

016; 176387; "Administration Study of E-187 in Rabbits During the Period of Organogenesis of Fetuses [II]" (Tanase, H.; Sankyo Company, Ltd., Laboratory Animal Science and Toxicology Laboratories, Fukuroi, Shizuoka, Japan; Report No.: TR135-132, 1/6/89). Fifteen to 20 mated females per group were treated orally via rubber catheter with 0, 5, 50, or 500 mg/kg/day of E-187 (Lot # MA-22-2; purity 95.7 %) from gestation days 6-18. Administration by gavage resulted in dose-related changes in the mean food consumption and mean body weight gain between gestation days 20-27. Marked reduction in food intakes and body weights were observed in 6/20 dams in the 500 mg/kg/day group. From the dams affected at 500 mg/kg/day: 1 dam died on gestation day 20; 1 had a still birth, and another had an abortion on gestation day 25; and 1 had dead fetuses at termination on gestation day 27. The fetal body weights were suppressed in the other 2/20 dams affected by treatment, however, the same level of suppression in fetal weights was also observed in dams from other doses and the control group. There were no significant differences in corpora lutea, implantations, # of live fetuses, or in the fetal mean body weights between the treated and control groups. Also, no significant differences in the frequency of external, skeletal and visceral anomalies were observed between the treated and controls. The fetal death and suppression of development in the affected dams at 500 mg/kg/day dose level were considered secondary to the marked reduction of their food uptake. **No adverse effects. Maternal NOEL: 50 mg/kg/day (based on reduction of food intake and body weight in 6/20 dams at 500 mg/kg/day dose level); **Developmental NOEL:** 500 mg/kg/day (based on lack of treatment-related effects observed at HDT); **Study Acceptable** (Eya, 12/04/00).

GENE MUTATION

** 019; 176390; "E-187: Microbial mutagenicity study"; (Ohta, T.; The Institute of Environmental Toxicology, Kodaira City, Tokyo 187, Japan.; Laboratory Project ID No. IET 85-0136; 09/08/86). E-187 technical (Lot # not given in text; purity ca. 93 % a.i.) was tested in the bacterial reverse mutation assay using *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and *E. coli* strain WP2 *uvr*A. The tests were conducted in the presence and absence of PCB-Aroclor 1254 induced rat liver S9. The tester strains were treated at 7-dose levels of E-187 ranging from 5 to 5000 ug/plate with and w/o activation. Each treatment level was plated in triplicate, and the assay was conducted twice to confirm the results for the reverse mutation assay. There were no reproducibly significant increases in the revertants at any dose level and in any strain. E-187 did not induce either base-pair or frame-shift mutations in any of the tester strains at doses up to 5000 ug/plate with or without added metabolic activation, positive controls functional. **No adverse effect indicated. Study Acceptable.** (Eya, 01/04/01).

**019; 176392; "Milbemectin: Mutation at the Thymidine Kinase (*tk*) Locus of Mouse Lymphoma L5178Y Cells (MLA) using the Microtitre^{R} Fluctuation Technique" (Fellows, M.; Covance Laboratories

Ltd., North Yorkshire, England, Laboratory Project I.D. No.: CLE Study # 730/9, 05/98). E-187 (Batch # 174903, 98 % purity) was assayed for its ability to induce mutation at the *tk* locus (5-trifluorothymidine resistance) in mouse lymphoma cells using a fluctuation protocol with 2 trials. Cultures without S9 (-S9) at E-187 concentrations of 0.234-30 ug/mL, and with S9 (+S9) at E-187 concentrations of 3.125-62.5 ug/mL, were selected for test for the mutation assay (p. 23). At 30 ug/mL, the relative survival rates were 13.09% (-S9) and 100.02% (+S9). No statistically significant increases in mutation frequency were observed following treatment with E-187 at any dose level tested in the absence or in presence of S9. Under the conditions employed in this study, E-187 was not mutagenic in this test system, positive controls functional. **No adverse effect indicated. Study Acceptable** (Increase in mutation was not observed at dose levels which were not toxic to the lymphoma cells with or without S9 activation) (Eya, 01/16/01).

CHROMOSOME EFFECTS

019; 176391; "E-187: *In vitro* cytogenetics test"** (Sasaki, Y.F.X.; The Institute of Environmental Toxicology, Kodaira City, Tokyo 187, Japan, Laboratory Project I.D. No.: IET 85-0137, 10/24/86). Duplicate cultures of Chinese hamster lung cells (CHL) at each dose were treated in medium containing 10% calf bovine serum without S9 at E-187 concentrations of 3.3, 10, 33, 58, and 100 µM for 24 and 48 hours, and with S9 at E-187 concentrations of 10, 33, 100, 330, and 1000 µM for 6 hours. E-187 of Lot # M-54 (A3:A4 = 3:7), and 93% purity was used for the test. One hundred metaphases were scored from each of the two replicate cultures at each dose level at each harvest time (24 and 48 hours, -S9; 18 and 24 hours, +S9). The frequencies of metaphases containing structural chromosome aberrations were < 5% in both systems. E-187 did not induce chromosomal aberrations in CHL cells in the presence and absence of metabolic activation, positive control functional . **No adverse effect indicated.** No increase was observed in the number of cells with aberrations at any of the dose levels with or without S9 activation. **Study Acceptable** (Eya, 01/12/01).

DNA DAMAGE

019; 176390; "E-187: Microbial mutagenicity study"; (Ohta, T.; The Institute of Environmental Toxicology, Kodaira City, Tokyo 187, Japan.; Laboratory Project ID No. IET 85-0136; 09/08/86). E-187 technical (Lot # not given in text; purity ca. 93 % a.i.) was tested in the DNA repair assay (Rec-assay) using *Bacillus subtilis* H17 (rec^+) and M45 (rec^-). The assays were done in the presence and absence of PCB-Aroclor 1254 induced rat liver S9. The tester strains were treated at 7-dose levels of test material ranging from 50 to 5000 ug/disk/plate with and w/o activation. DNA repair test was plated once and was not repeated to confirm the results (or results from the replicates were not presented in the text). There were no significant differences in growth at doses of E-187 up to 5000 ug/plate, with or without added metabolic activation. **No adverse effect indicated. Study Unacceptable but possibly upgradeable** with the following information: results from more than one trial to confirm the data; preparation, storage and phenotype verification of stock culture or bacteria; solvents used for positive and negative control substances; how the zone of inhibition was established; and viability data of the tester strains (Eya, 01/04/01).

**019; 176393; "Milbemectin: Induction of Micronuclei in the Bone Marrow of Treated Mice" (Burman, M.; Covance Laboratories Ltd., North Yorkshire, England, CLE Study No. 730/10, 06/98). Five mice/group were dosed with (M): 0, 25, 50, and 100 mg/kg and (F): 0, 37.5, 75 and 150 mg/kg of E-

187 (Lot # 174903, 98.01% purity) orally once daily on two consecutive days. The mice were sacrificed 24 or 48 hours after the second administration. Several animals (F: 3/15) receiving the highest dose died prior to scheduled sacrifice. The test material at dose volume of 20 mL/kg was prepared in 0.5% carboxymethyl cellulose (CMC). Mice treated with E-187 at all doses exhibited group mean ratios of PCE to NCE and frequencies of micronucleated PCE which were comparable to the values from vehicle controls at both sampling times. E-187 did not induce micronuclei in the polychromatic erythrocytes of the bone marrow of mice treated up to 100 mg/kg/day (M) and 150 mg/kg/day (F). Based on these results, E-187 was concluded to be negative for causing cytogenetic damage as evaluated by micronucleus induction. Death of (F): 3/15 at 150 mg/kg (main study), and (M): 2/3 at 150 mg/kg (preliminary study). **No adverse effect indicated. Study Acceptable.** (Eya, 01/23/01).

NEUROTOXICITY

009; 176378; "Acute Oral Gavage Neurotoxicity Study with Milbemectin in Rats" (Weiler, M.S., Covance Laboratories Inc., Madison, WI, Laboratory Project Identification: Covance 6402-223, 6/19/98). 818. Milbemectin Technical (Lot No. 174903, purity = 98.01%), suspended in a 0.5% solution (w/v) of carboxymethylcellulose in reverse osmosis water, was administered by gavage in a single dose to 10 Crl:CD® (SD)BR VAF/Plus® rats per sex per dose at dose levels of 0 (vehicle only), 20, 100, 500 (10 males, 7 females), and 60 (8 females only) mg/kg. No male animals died. Female mortalities occurred as follows: 0/10, 0/10, 1/10, 7/7, 0/8, respectively. Treatment-related clinical signs observed included tremors, ataxia, hypoactivity, and irregular respiration; only at 20 mg/kg were no clinical signs observed. All treatment-related clinical signs cleared in all surviving animals after Day 2. FOB assessments conducted on Day 1 revealed treatment-related decreases in forelimb strength (during the second trial) and body temperature in males at 500 mg/kg. No treatment-related effects were observed during FOB assessments conducted on Days 8 and 15. Motor activity assessments revealed a dose-related decrease in the mean number of counts beginning at 20 mg/kg. Microscopic examination of preserved nervous system tissues revealed no treatment-related abnormalities. **Possible adverse effect:** entire body tremors. NOEL (M/F)< 20 mg/kg (based on a treatment-related decrease in motor activity). **Acceptable.** (Corlett and Leung, 1/24/01)

013; 176382; "13-Week Dietary Neurotoxicity Study with Milbemectin in Rats" (Weiler, M.S., Covance Laboratories Inc., Madison, WI, Laboratory Project Identification: Covance 6402-224, 6/19/98). 827. Milbemectin Technical (Lot No. 174903, purity = 98.01%) was admixed to the diet at dose levels of 0 (basal diet only), 150, 375, or 750 ppm (for males, 0, 12.3, 32.0, and 59.4 mg/kg/day, respectively, and for females, 0, 13.4, 35.6, and 72.4 mg/kg/day, respectively) and fed to 10 Crl:CD® (SD)BR VAF/Plus® rats per sex per dose daily for 13 weeks. No animals died. No treatment-related clinical signs were observed. FOB and motor activity assessments revealed no treatment-related effects. Microscopic examination of preserved nervous system tissues revealed no treatment-related abnormalities. **No adverse effects.** NOEL (M)= 59.4 mg/kg/day (750 ppm) and NOEL (F)=72.4 mg/kg/day (750 ppm) based on no effects at highest dose tested. **Unacceptable but possibly upgradeable** with the submission of the justification for dose level selection. (Corlett and Leung, 1/26/01)

SUBCHRONIC STUDIES

(Oral)

010; 176379; "Thirteen-Week Consecutive Administration Study of E-187 in Rats Through Diet" (Masuda, H., Sankyo Company, Ltd., Laboratory Animal Science and Toxicology Laboratories, Fukuroi

City, Shizuoka Prefecture, Japan, Report No. TR133-068, 8/14/86). 821. E-187 (Lot No. M-83, purity = 92.9%) was admixed to the diet at dose levels of 0 (basal diet only), 375, 750, 1500, or 3000 ppm (for males, 0, 24.96, 49.09, 100.70, and 213.48 mg/kg/day, respectively, and for females, 0, 27.80, 55.74, 116.09, and 230.84 mg/kg/day, respectively) and fed to 10 F344 rats per sex per dose daily for 13 weeks. No animals died. At 3000 ppm, stained eyelids, hypersensitivity, staggering, piloerection, decreased movement, and abnormal growth of the upper and lower front teeth were reported in both sexes after the initiation of exposure with stained eyelids, hypersensitivity, decreased movement, and abnormal tooth growth persisting until the end of the study. Stained eyelids were reported in both sexes at 1500 ppm. A treatment-related decrease in body weight was observed in both sexes at 3000 ppm. Treatment-related decreases in mean hemoglobin and hematocrit levels in males beginning at 1500 ppm and in females beginning at 750 ppm were observed. Treatment-related decreases in mean corpuscular volume and mean corpuscular hemoglobin levels in males beginning at 750 ppm and in females beginning at 375 ppm were observed. A treatment-related increase in the red blood cell count in females beginning at 750 ppm and a treatment-related increase in reticulocytes in both sexes at 3000 ppm were observed. Microscopic examination revealed treatment-related hypertrophy of liver cells (both sexes at 1500 and 3000 ppm), a treatment-related acceleration of hematopoiesis in the spleen (both sexes at 3000 ppm) and in the bone marrow (both sexes at 1500 and 3000 ppm), and cellular hypertrophy in the zona fasciculata of the adrenal glands (both sexes at 1500 and 3000 ppm). **No adverse effects.** NOEL (M)= 24.96 mg/kg/day (375 ppm) and NOEL (F)< 27.80 mg/kg/day (375 ppm) based on decreased mean corpuscular hemoglobin and mean corpuscular volume levels. **Unacceptable but possibly upgradeable** with the submission of 1) data confirming the stability of the dosing material and 2) observations of individual test animals. (Corlett, 2/1/01)

011; 176380; "E-187: 13-Week Oral Toxicity Study in Dogs" (Ebino, K., Mitsukaido Laboratories, The Institute of Environmental Toxicology, Kodaira City, Tokyo, Japan, Laboratory Project ID: IET 86-0123, 1/13/88). 821. E-187 (Lot No. MA-22, purity = 93.7%) was administered to 4 beagle dogs per sex per dose by gelatin capsule at dose levels of 0 (empty capsule), 3, 10, or 30 mg/kg/day once a day 7 days a week for 13 weeks. No animals died. Treatment-related tremor of the head, sedation, salivation, staggering gait, and eye discharge were observed in both sexes at 30 mg/kg/day. A treatment-related decrease in body weight and a treatment-related increase in mean relative adrenal weight were observed in males at 30 mg/kg/day. Microscopic examination revealed treatment-related C-cell hyperplasia in females at 30 mg/kg/day. **Possible adverse effect**: tremor of the head. NOEL (M/F)= 10 mg/kg/day based on clinical signs. **Acceptable**. (Corlett, 2/5/01)

(Dermal)

012; 176381; "Milbemectin-28-Day Dermal Toxicity Study in Rabbits" (Kuhn, J.O., Stillmeadow Incorporated, Sugar Land, TX, Laboratory Study No. 5302-99, 2/24/00). 822. Milbemectin Technical (Lot No. 590940, purity = 98.24%), moistened with deionized water, was applied to the clipped skin of 5 New Zealand White rabbits per sex per dose at dose levels of 0 (sham-treated animals), 100, 500, or 1000 mg/kg/day (5 additional animals per sex at 0 and 1000 mg/kg/day served as satellite groups) for 6 hours per day, 7 days a week for 28 days. No animals died. No treatment-related clinical signs were observed. No treatment-related body weight, hematological, or serum chemistry effects were observed.

Macroscopic and microscopic examinations revealed no treatment-related abnormalities. **No adverse effects.** NOEL (M/F, systemic and skin)= 1000 mg/kg/day based on no effects at the highest dose tested. **Unacceptable and not upgradeable** because no ophthalmological examinations were conducted on the test animals. (Corlett, 2/7/01)

METABOLISM STUDIES

020; 176394; "Metabolic Study of E-187 in Rats" (Sadakane, S., Ando, M., Tanaka, K., Sankyo Company Ltd., Agroscience Research Laboratories, Shiga 520-2342, Japan, Report No.: AM 90-005, 04/19/90). Fischer rats (3/sex/group) were dosed with ^{14}C-E-187 orally by gavage at dose levels of 2.5, 25 or 250 mg/kg according to the following regimen: (1) single administration of a mixture 5-^{3}H-A3: ^{14}C-A4 (3:7), at 2.5 and 25 mg/kg body weight; (2) single administration of 5-^{3}H-A3 (250 mg/kg); single administration ^{14}C-A3 (25 mg/kg); single administration ^{14}C-A4 (25 mg/kg); and consecutive administration ^{14}C-A4 for 10 days at 2.5 mg/kg/day. The elimination patterns of E-187 indicated that > 95% in 3 days and > 98% in 7 days were excreted after administration. Most of the radioactivity was excreted in feces, and urine. Maximum concentration in blood was reached in 3 hours with t ½ of 7-8 hours. Liver and fat had the highest level of radioactivity compared to other organs and tissues. A3 was eliminated/degraded faster than A4. Accumulation of E-187 was not observed in any organ or tissue. The degradation pathway of E-187 was hydroxylation on the C13, C23, C26, C27, C28, C29 and C30 positions of the molecule, followed by further oxidation of hydroxyl groups to epoxide and ketone. The metabolites of highest concentration in the urine were 13,23-dihydroxyA4 and 13,30-dihydroxyA4, and in feces were A4, 13-hydroxyA4, dihydroxyA4s, and trihydroxyA4s. **Study Unacceptable,** details concerning experimental plan, data certifying the identity and purity of starting materials, analysis of dosing material, and quality assurance of the report were missing. (Eya, 01/29/01)

020; 176395; "Metabolism of ^{14}C-Milbemycin A_4 in Rats" (Fathulla, R., Ampofo, S., Georgeson, S., Covance Laboratories Ltd., Madison, WI, Laboratory Project ID No.: Covance 6402-219, 03/09/00). Fifty-three Fischer 344 rats (5/sex/group) were dosed with ^{14}C-Milbemycin A_4 (^{14}C-E187) orally by gavage at dose levels of 2.5 mg/kg (low dose) and 25 mg/kg (high dose) according to the following regimen: (1) single low dose; (2) 14-day repeated nonlabelled dose followed by a single ^{14}C low dose; (3) single high dose; (4) single low dose to monitor pharmacokinetics; and (5) single high dose for pharmacokinetics. Tissue distribution studies were conducted using 9 rats/sex/group by administering single oral low and high doses. Bile cannulation studies were conducted using 4 rats/sex/group by administering single oral low and high doses. For the single oral low and high dose and repeated dose groups, the mean total recoveries of the administered ^{14}C ranged from 93.7-106%, with 81.5-100% eliminated in feces and 3.28-9.29% in urine. The amount in the remaining tissues were $\leq$ 0.44% of the total radioactive residue (TRR). Excretion of ^{14}C was rapid with most being excreted within 24 hours of administration. The amount of ^{14}C detected in bile and urine indicated that ca. 50% (at low dose) and 30% (at high dose) of ^{14}C was absorbed. Following the oral dose, the plasma concentration of ^{14}C peaked after 2-3 hours post-dose. Most of the ^{14}C in tissues was found in the gastrointestinal tract or in the liver. The major metabolite found in plasma, liver, and kidney samples was 13-hydroxyA4, accounting for up to 60.2, 52.4, and 66.7% of TRR, respectively. The following metabolites were characterized from the feces: dihydroxylated A4s (3 metabolites); and trihydroxylated A4s (2 metabolites). The major urinary metabolite was dihydroxylated A4s (0.95-4.38% of administered dose). **Study Acceptable.** (Eya, 01/31/01)